ABSTRACT OF THE DISCLOSURE

The present invention provides a new protocol for quantifying multiplex real-time polymerase chain reaction (PCR). In particular, the present invention provides methods of quantifying multiple PCR products or amplicons in a single real-time PCR reaction based on the different melting temperatures (T_m) of each amplicon and the emission changes of double stranded DNA dyes such as SYBR Green I when amplicons are in duplex or in separation. For a specific amplicon with a T_m, the emission difference between the emission reading taken at a temperature below the T_m and the emission reading taken at a temperature above the T_m corresponds to the emission value of the amplicon in duplex. Accordingly, the emission difference of each amplicon in a single PCR reaction can be used to quantify each amplicon. The present invention further provides computer programs or computer products which perform the methods described herein.